

What is the future of electrical impedance spectroscopy in flow cytometry?

Cite as: Biomicrofluidics **15**, 061302 (2021); doi: 10.1063/5.0073457

Submitted: 30 September 2021 · Accepted: 23 November 2021 ·

Published Online: 6 December 2021



View Online



Export Citation



CrossMark

Furkan Gökçe,^{a)} Paolo S. Ravaynia, Mario M. Modena, and Andreas Hierlemann

AFFILIATIONS

Bioengineering Laboratory, Department of Biosystems Science and Engineering, ETH Zürich, Mattenstrasse 26, 4058 Basel, Switzerland

^{a)}**Author to whom correspondence should be addressed:** furkan.gokce@bsse.ethz.ch

ABSTRACT

More than 20 years ago, electrical impedance spectroscopy (EIS) was proposed as a potential characterization method for flow cytometry. As the setup is comparably simple and the method is label-free, EIS has attracted considerable interest from the research community as a potential alternative to standard optical methods, such as fluorescence-activated cell sorting (FACS). However, until today, FACS remains by and large the laboratory standard with highly developed capabilities and broad use in research and clinical settings. Nevertheless, can EIS still provide a complement or alternative to FACS in specific applications? In this Perspective, we will give an overview of the current state of the art of EIS in terms of technologies and capabilities. We will then describe recent advances in EIS-based flow cytometry, compare the performance to that of FACS methods, and discuss potential prospects of EIS in flow cytometry.

Published under an exclusive license by AIP Publishing. <https://doi.org/10.1063/5.0073457>

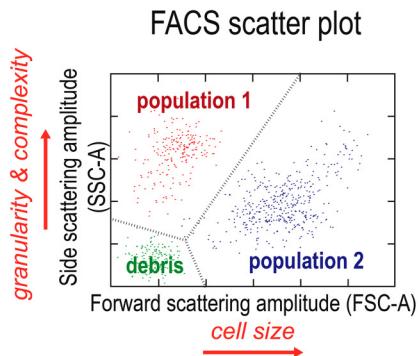
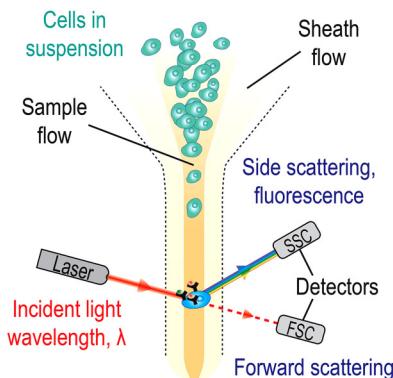
INTRODUCTION

In the early 2000s, electrical impedance spectroscopy (EIS) was introduced in flow cytometry for multi-parametric analysis of cells in suspension.^{1,2} The interest in EIS was fueled by its potential to become a low-complexity and low-cost alternative to established fluorescence- and scattering-based detection methods, which were commonly employed in flow cytometry, the so-called fluorescence-activated cell sorting (FACS) [Fig. 1(a)]. Inspired by the Coulter counter principle,³ EIS-based detection methods for flow cytometry (EIS-FC) measure variations in electrical impedance, while a flow of medium including cells passes over or in between a set of electrodes in contact with the suspension medium.^{2,4,5} EIS-FC uses a microfluidic channel to align the cells for single-cell detection and to focus them within the sensing volume, an approach very similar to that employed for focusing cells in the detection volume of a FACS cytometer [Fig. 1(b)]. The microfluidic channel provides a small sample volume between the electrodes ($\sim 10 \text{ nl}$) for increased sensitivity, and the laminar flow profile enables us to precisely control the cell flow.^{1,6} Similar information on the cells in suspension can be obtained by using either label-free FACS-based or EIS-based flow cytometry. Label-free FACS analysis relies on the detection of forward- and side-scattered light (FSC-A and SSC-A) to get information on cell size and cell

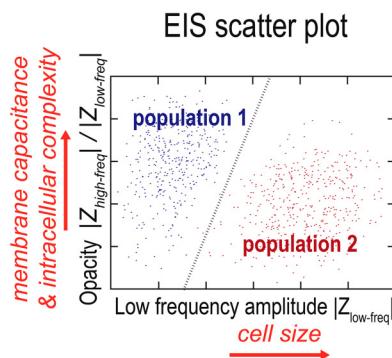
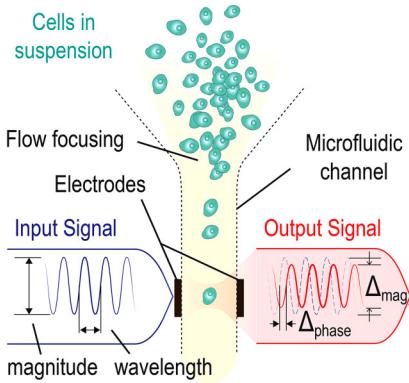
granularity [Fig. 1(a)]. By recording cell impedance at multiple frequencies to comprehensively assess cell dielectric properties, EIS-FC provides estimates of the cell size at low frequencies (usually around or below 100 kHz) or information on membrane integrity and intracellular content by looking at the impedance at high frequencies (from $\sim 100 \text{ kHz}$ to $\sim 10\text{--}100 \text{ MHz}$) [Fig. 1(c)].² High-frequency impedance recordings are then often normalized with respect to low-frequency impedance values to be able to compare information on the membrane integrity of cells that feature different geometric dimensions or sizes. The ratio of high-frequency impedance to low-frequency impedance values is referred to as “opacity” [Fig. 1(b)].^{2,7,8}

When EIS-FC was initially developed, FACS instruments required frequent re-alignment of the optical elements and highly trained personnel for data acquisition and analysis. In contrast, the sensing electrodes of EIS-FC could be integrated within the microfluidic channel, so that no user alignment was necessary. Furthermore, EIS signal detection could be carried out with small and portable electronic equipment, which resulted in a small footprint of the detection system and low maintenance and acquisition costs. Therefore, EIS-FC initially raised considerable interest in the scientific community, and many implementations have been developed with the aims to increase sensitivity, simplify electrode

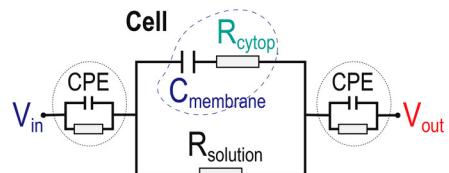
A) FACS flow cytometry



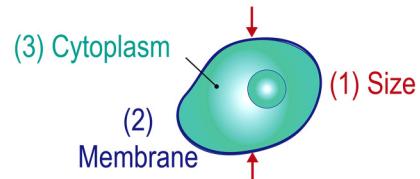
B) EIS-based flow cytometry



C) EIS electrical equivalent circuit



CPE: Constant-phase elements representing the EIS electrodes



Impedance signal (vs. frequency)

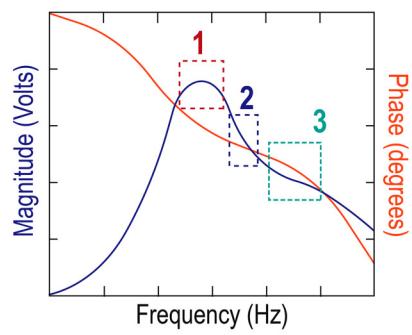


FIG. 1. Working principles of (a) FACS-based flow cytometry and (b) EIS-based flow cytometry, showing the application and recording of electrical AC signals. In the sub-panels are typical FACS and EIS scatterplots of a hypothetical mixture of two populations, which show similarities for label-free analysis. In a typical FACS scatterplot, debris is visible besides the cell populations, while it is generally not plotted in EIS scatterplots. Opacity, as shown along the y axis of the EIS scatterplot, denotes the ratio of the high- and low-frequency signal amplitude. It is used to minimize dependence of EIS signals on the relative positions of the measured cells. Opacity can be used to characterize membrane integrity and intracellular contents of the cells. (c) Top: equivalent-circuit model of the sensing region, showing the contributions of the electrodes, of the solution, and of the passing cell to the sensed impedance. Constant phase elements (CPE) represent the electrodes. Bottom: signal-magnitude and phase-response plots of cells in dependence of the applied frequency.

integration, and extend the information that can be obtained through EIS by using refined electrical-equivalent models of cells in an electrolyte flow.^{5,9–11}

IS EIS-BASED FLOW CYTOMETRY A VIABLE ALTERNATIVE TO FACS?

EIS-FC has proven to be competitive with FACS-based cytometry in multiple aspects. By using state-of-the-art electronic components and by parallelizing the analysis, EIS-FC can readily be used to record thousands of events per second,^{10,12,13} which matches the

throughput of FACS-based analysis.¹⁴ FACS detection has been optimized for the analysis of single mammalian cells in the 1–100 μm diameter range,¹⁴ while *ad hoc* modifications of the instrumentation can be made to extend the measurement range from sub-micrometer^{15–17} to millimeter-range particles.^{18,19} However, such modifications require significant efforts and are costly. In contrast, EIS detection can be easily adapted for a wide range of dimensions of analyzed particles—from sub-micrometer particles (e.g., extracellular vesicles or apoptotic bodies) to large cell aggregates or organisms (e.g., organoids or parasites)—with minimal modifications to the measurement equipment. Only comparably

simple modifications of the detection chamber and a careful selection of the frequency range of the impedance signals that will be recorded are required.^{20–27}

The main limitation of EIS-FC originates from its limited capability in identifying different cell types in a mixed population as a consequence of the label-free nature of cell-size and membrane-property measurements. In contrast, great specificity is provided by fluorescent labels of different cellular markers that enable us to characterize mixed cell populations at high resolution by using FACS. FACS techniques have experienced drastic improvements in instrumentation and in the development of novel labeling strategies for fluorescence-based cell characterization,²⁸ which have greatly improved its performance and user-friendliness. New-generation FACS instruments require minimal or no alignment of the optical elements and can be readily used for automated high-throughput detection. Furthermore, a comprehensive analysis can now be carried out with only a few microliters of the sample. Modern FACS devices that include multiple laser sources can simultaneously detect up to 20 parameters for characterizing and differentiating cells in mixed populations. The use of quantum-dot-based cellular labels could enable FACS detection by using up to ~50 different markers.¹⁴ The analysis of these multidimensional FACS data has also been greatly facilitated by the development of intuitive and automated analysis software packages. The integration of machine-learning approaches for automated clustering is expected to further improve the analysis performance and simplify data interpretation.^{14,29} Improvements in FACS technology have promoted its adoption in research, industrial, and clinical settings despite the high initial costs to purchase the equipment.^{12,14,30,31} A disruptive recent advancement in flow cytometry includes the integration of high-speed cameras for high-resolution imaging in FACS flow cytometers.^{32–34} This new method, which has been termed image-activated cell sorting (IACS) or imaging flow cytometry (IFC), provides spatial information on the distribution of labeled markers in single cells in addition to the characterization by FACS-based analysis. IACS provides more information and higher data dimensionality and enables in-depth characterization and refined differentiation of cells in mixed populations. An

overview of the most important features of EIS-FC, FACS, and IACS is given in Table I.

Although the application of EIS-FC requires significantly less investments in terms of setup costs and efforts for operation, this electrical analysis method has been commercially developed to a much lesser extent than optical methods and has been applied mostly to cell counting.³⁵ Meanwhile, FACS devices and FACS analysis have been developed at a fast pace and are broadly available to researchers and clinicians. Therefore, an obvious question concerns the potential future of EIS-based detection methods in flow cytometry.

WHAT IS THE FUTURE OF EIS-BASED FLOW CYTOMETRY?

Flow cytometry is predominantly used as an end point analysis method to characterize and analyze cell populations, where the performance of FACS analysis is superior to that of EIS-FC. The single-cell resolution of flow cytometry methods could, however, also provide a wealth of information in cell cultures and could be used to monitor dynamic changes in real time. The large form factor of FACS equipment prevents integration in compact systems and cell-culture platforms. In contrast, EIS methods could be an ideal candidate for continuous characterization of a wide range of biological samples, as they are conveniently configurable, label-free, and can easily be automated and multiplexed.^{24,26} Application examples include monitoring responses upon drug exposure. The parallel and continuous EIS monitoring of cancer microtissues upon exposure to drug compounds was recently demonstrated.²¹ The microtissues were periodically moved across EIS electrodes by simple tilting of the culture platform, which initiated medium flow and enabled parallel impedance screening of multiple microtissues in real time. EIS-FC could potentially be used in a similar way for label-free real-time monitoring of suspended cells at single-cell resolution.

Commercial impedance-based Coulter counter systems have seen great technological improvements in the last few years and feature high throughput and a large detection range spanning from

TABLE I. Summary of the main features of EIS-FC, FACS, and IACS.

	EIS-FC	FACS	IACS
Installation costs	<\$100k	\$50k–\$1M	>\$1M
Throughput in events per second	Tens of thousands of cells per second	Tens of thousands of cells per second	Around 100 cells per second
Dimensionality of data	Defined by the number of applied and recorded electrical signal frequencies	Around 20 features for a standard FACS device	High-dimensional data due to spatial information provided through imaging
Integration and multiplexing	Convenient integration and scalability due to electronic multiplexing	Limited and expensive due to setup costs and optical alignment	Limited due to complex optics, setup costs, and optical alignment
Label-free	Yes	Yes + fluorescence	Yes + fluorescence
Real-time or end point	Both	Limited to end point	Limited to end point

sub-micrometer particle diameters to a few millimeters. They have been used for the characterization of a broad variety of samples including body fluids.^{36–39} The label-free electrical detection principle obviates long sample preparation times and the need for expensive dyes, which renders EIS systems particularly interesting for diagnostic and medical purposes. Academic research efforts have yielded micro-fabricated devices with distinct advantages over commercial impedance-based cytometers, which include reduced sample volumes, low power consumption, and small system dimensions and portability.⁴⁰

The compact nature and high level of integration of EIS-FCs also enable application as in-line analysis techniques for quality control or monitoring of cell-manipulation protocols in systems with integrated electrodes. For instance, EIS-FC could be used to monitor the effectiveness of cell electroporation in flow-through electroporators or for optimization of electroporation parameters.^{41,42} Similarly, integrated electrodes for EIS-FC could be utilized in droplet microfluidics for sorting and analyzing encapsulated cells^{43–45} or for manipulating droplets.^{46,47} Finally, EIS-FC could also find applications in quality assessment of bio-printing of cells and organoids to verify, in an automated and high-throughput manner, size uniformity and viability.^{48–50}

CONCLUSION

In conclusion, although EIS can still be used as an end point analysis method in flow cytometry as suggested two decades ago, the technological advances in FACS and the recent introduction of optical microscopy information in IACS flow cytometry render the optical techniques superior for cell population measurements. However, the label-free nature and high flexibility in configuring and integrating EIS methods may open a variety of applications for EIS methods. Examples include the real-time monitoring of compound effects in long-term drug exposure experiments or the in-line monitoring of cells and organoids for quality control purposes during bio-printing. Finally, the possibility of realizing small-outline and low-cost instrumentation renders EIS-based methods to be very attractive in resource-limited settings or for analyzing body fluids in the growing field of wearable medical devices.

ACKNOWLEDGMENTS

The authors thank Dr. Aleksandra Gumienny for her valuable input on FACS. This work was financially supported by the Swiss National Science Foundation under Contract No. CR32I2_166329: “Infected body-on-chip.” F.G. acknowledges individual support through the Personalized Health and Related Technologies (PHRT) initiative of the ETH Domain (Project No. SFA-PHRT-2017-309).

AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

REFERENCES

- ¹H. E. Ayliffe, A. B. Frazier, and R. D. Rabbitt, “Electric impedance spectroscopy using microchannels with integrated metal electrodes,” *J. Microelectromech. Syst.* **8**, 50–57 (1999).
- ²S. Gawad, L. Schild, and Ph. Renaud, “Micromachined impedance spectroscopy flow cytometer for cell analysis and particle sizing,” *Lab Chip* **1**, 76 (2001).
- ³W. H. Coulter, “Means for counting particles suspended in a fluid,” U.S. patent 2,656,508 (20 Oct 1953).
- ⁴S. Gawad, K. Cheung, U. Seger, A. Bertsch, and P. Renaud, “Dielectric spectroscopy in a micromachined flow cytometer: Theoretical and practical considerations,” *Lab Chip* **4**, 241 (2004).
- ⁵T. Sun and H. Morgan, “Single-cell microfluidic impedance cytometry: A review,” *Microfluid. Nanofluid.* **8**, 423–443 (2010).
- ⁶K. C. Cheung *et al.*, “Microfluidic impedance-based flow cytometry,” *Cytometry Part A* **77A**, 648–666 (2010).
- ⁷W. H. Coulter and W. R. A. Hogg, “Collating apparatus for pairs of electrical pulses produced by particle analyzing apparatus,” U.S. patent 3,502,973 (24 March 1970).
- ⁸R. A. Hoffman, T. S. Johnson, and W. B. Britt, “Flow cytometric electronic direct current volume and radiofrequency impedance measurements of single cells and particles,” *Cytometry* **1**, 377–384 (1981).
- ⁹S. Zhu *et al.*, “Microfluidic impedance cytometry for single-cell sensing: Review on electrode configurations,” *Talanta* **233**, 122571 (2021).
- ¹⁰C. Honrado, P. Bisegna, N. S. Swami, and F. Caselli, “Single-cell microfluidic impedance cytometry: From raw signals to cell phenotypes using data analytics,” *Lab Chip* **21**, 22–54 (2021).
- ¹¹C. Petchakup *et al.*, “Advances in single cell impedance cytometry for biomedical applications,” *Micromachines* **8**, 87 (2017).
- ¹²R.-J. Yang, L.-M. Fu, and H.-H. Hou, “Review and perspectives on microfluidic flow cytometers,” *Sens. Actuators B Chem.* **266**, 26–45 (2018).
- ¹³Z. Liao *et al.*, “Recent advances in microfluidic chip integrated electronic biosensors for multiplexed detection,” *Biosens. Bioelectron.* **121**, 272–280 (2018).
- ¹⁴S. M. Manohar, P. Shah, and A. Nair, “Flow cytometry: Principles, applications and recent advances,” *Bioanalysis* **13**, 185–198 (2021).
- ¹⁵C. P. D. Brussaard, D. Marie, and G. Bratbak, “Flow cytometric detection of viruses,” *J. Virol. Methods* **85**, 175–182 (2000).
- ¹⁶H. B. Steen, “Flow cytometer for measurement of the light scattering of viral and other submicroscopic particles,” *Cytometry* **57A**, 94–99 (2004).
- ¹⁷G. J. A. Arkesteyn, E. Lozano-Andrés, S. F. W. M. Libregts, and M. H. M. Wauben, “Improved flow cytometric light scatter detection of submicron-sized particles by reduction of optical background signals,” *Cytometry Part A* **97**, 610–619 (2020).
- ¹⁸D. A. Watson, D. F. Gaskell, L. O. Brown, S. K. Doorn, and J. P. Nolan, “Spectral measurements of large particles by flow cytometry,” *Cytometry Part A* **75A**, 460–464 (2009).
- ¹⁹C. J. Randall *et al.*, “Rapid counting and spectral sorting of live coral larvae using large-particle flow cytometry,” *Sci. Rep.* **10**, 12919 (2020).
- ²⁰N. Haandbæk, S. C. Bürgel, F. Heer, and A. Hierlemann, “Characterization of subcellular morphology of single yeast cells using high frequency microfluidic impedance cytometer,” *Lab Chip* **14**, 369–377 (2014).
- ²¹S. C. Bürgel, L. Diener, O. Frey, J.-Y. Kim, and A. Hierlemann, “Automated, multiplexed electrical impedance spectroscopy platform for continuous monitoring of microtissue spheroids,” *Anal. Chem.* **88**, 10876–10883 (2016).
- ²²N. Haandbæk, O. With, S. C. Bürgel, F. Heer, and A. Hierlemann, “Resonance-enhanced microfluidic impedance cytometer for detection of single bacteria,” *Lab Chip* **14**, 3313–3324 (2014).
- ²³C. Honrado *et al.*, “Apoptotic bodies in the pancreatic tumor cell culture media enable label-free drug sensitivity assessment by impedance cytometry,” *Adv. Biol.* **5**, 2100438 (2021).
- ²⁴D. C. Spencer *et al.*, “A fast impedance-based antimicrobial susceptibility test,” *Nat. Commun.* **11**, 5328 (2020).

- ²⁵P. S. Ravaynia *et al.*, “Parallelized impedance-based platform for continuous dose-response characterization of antischistosomal drugs,” *Adv. Biosyst.* **4**, 1900304 (2020).
- ²⁶K. Chawla *et al.*, “Integrating impedance-based growth-rate monitoring into a microfluidic cell culture platform for live-cell microscopy,” *Microsyst. Nanoeng.* **4**, 1–12 (2018).
- ²⁷W. Tang, D. Tang, Z. Ni, N. Xiang, and H. Yi, “Microfluidic impedance cytometer with inertial focusing and liquid electrodes for high-throughput cell counting and discrimination,” *Anal. Chem.* **89**, 3154–3161 (2017).
- ²⁸K. M. McKinnon, “Flow cytometry: An overview,” *Curr. Protoc. Immunol.* **120**, 5.1.1 (2018).
- ²⁹Y. Gong, N. Fan, X. Yang, B. Peng, and H. Jiang, “New advances in microfluidic flow cytometry,” *Electrophoresis* **40**, 1212–1229 (2019).
- ³⁰D. L. Jaye, R. A. Bray, H. M. Gebel, W. A. C. Harris, and E. K. Waller, “Translational applications of flow cytometry in clinical practice,” *J. Immunol.* **188**, 4715–4719 (2012).
- ³¹M. Doan *et al.*, “Diagnostic potential of imaging flow cytometry,” *Trends Biotechnol.* **36**, 649–652 (2018).
- ³²N. Nitta *et al.*, “Intelligent image-activated cell sorting,” *Cell* **175**, 266–276.e13 (2018).
- ³³A. Isozaki *et al.*, “A practical guide to intelligent image-activated cell sorting,” *Nat. Protoc.* **14**, 2370–2415 (2019).
- ³⁴L. E. Weiss *et al.*, “Three-dimensional localization microscopy in live flowing cells,” *Nat. Nanotechnol.* **15**, 500–506 (2020).
- ³⁵A. Vembadi, A. Menachery, and M. A. Qasaimeh, “Cell cytometry: Review and perspective on biotechnological advances,” *Front. Bioeng. Biotechnol.* **7**, 147 (2019).
- ³⁶E. M. Strohm *et al.*, “Sizing biological cells using a microfluidic acoustic flow cytometer,” *Sci. Rep.* **9**, 4775 (2019).
- ³⁷C. Martinelli *et al.*, “Development of artificial plasma membranes derived nanovesicles suitable for drugs encapsulation,” *Cells* **9**, 1626 (2020).
- ³⁸M. J. A. Martín *et al.*, “Automated cell count in body fluids: A review,” *Adv. Lab. Med. Av. Med. Lab.* **2**, 149–161 (2021).
- ³⁹I. Heidmann, G. Schade-Kampmann, J. Lambalk, M. Ottiger, M. D. Berardino, and T. Wang, “Impedance flow cytometry: A novel technique in pollen analysis,” *PLoS One* **11**, e0165531 (2016).
- ⁴⁰A. Furniturewalla, M. Chan, J. Sui, K. Ahuja, and M. Javanmard, “Fully integrated wearable impedance cytometry platform on flexible circuit board with online smartphone readout,” *Microsyst. Nanoeng.* **4**, 1–10 (2018).
- ⁴¹Y. Ye *et al.*, “Single-cell electroporation with real-time impedance assessment using a constriction microchannel,” *Micromachines* **11**, 856 (2020).
- ⁴²L. Buchmann, W. Frey, C. Gusbeth, P. S. Ravaynia, and A. Mathys, “Effect of nanosecond pulsed electric field treatment on cell proliferation of microalgae,” *Bioresour. Technol.* **271**, 402–408 (2019).
- ⁴³N. E. Yakdi, F. Huet, and K. Ngo, “Detection and sizing of single droplets flowing in a lab-on-a-chip device by measuring impedance fluctuations,” *Sens. Actuators B Chem.* **236**, 794–804 (2016).
- ⁴⁴J. Panwar and R. Roy, “Integrated field’s metal microelectrodes based microfluidic impedance cytometry for cell-in-droplet quantification,” *Microelectron. Eng.* **215**, 111010 (2019).
- ⁴⁵W. Fan *et al.*, “Single-cell impedance analysis of osteogenic differentiation by droplet-based microfluidics,” *Biosens. Bioelectron.* **145**, 111730 (2019).
- ⁴⁶A. J. T. Teo *et al.*, “Controllable droplet generation at a microfluidic T-junction using AC electric field,” *Microfluid. Nanofluid.* **24**, 1–9 (2020).
- ⁴⁷S. R. Doonan and R. C. Bailey, “K-channel: A multifunctional architecture for dynamically reconfigurable sample processing in droplet microfluidics,” *Anal. Chem.* **89**, 4091–4099 (2017).
- ⁴⁸J. Schoendube, D. Wright, R. Zengerle, and P. Koltay, “Single-cell printing based on impedance detection,” *Biomicrofluidics* **9**, 014117 (2015).
- ⁴⁹L. Gong *et al.*, “Direct and label-free cell status monitoring of spheroids and micr carriers using microfluidic impedance cytometry,” *Small* **17**, 2007500 (2021).
- ⁵⁰G. Hong *et al.*, “Production of multiple cell-laden microtissue spheroids with a biomimetic hepatic-lobule-like structure,” *Adv. Mater.* **33**, 2102624 (2021).